Functional Properties of Cowpea (*Vigna unguiculata*) Flour As Affected by Soaking, Boiling, and Fungal Fermentation

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Functional properties of cowpea flour as affected by soaking, soaking/boiling (S/B), and fermentation of seeds with *Rhizopus microsporus* subsp. *oligosporus* before milling were investigated. Soaking and fungal fermentation had less influence on functionality of flour compared to S/B, which markedly decreased solubility and impaired emulsifying properties. Solubility of heat-denatured proteins was slightly improved by fermentation. High emulsion capacity was not associated with high emulsion viscosity or solubility. The combined effects of heat treatment and fungal fermentation on equilibrium moisture content were apparent at 75−97% equilibrium relative humidity. Cowpea flours were consistently more hydrophilic than lipophilic, regardless of processing treatment. The least gelation capacity of flours increased as a result of heat treatment.

Keywords: *Cowpea flour; Vigna unguiculata; functional properties; soaking; heat treatment; fungal fermentation; Rhizopus microsporus subsp. Oligosporus*

INTRODUCTION

Cowpeas [*Vigna unguiculata* (L.) Walp], also known as black-eyed peas, Southern peas, and crowder peas, are underutilized in the United States and other industrialized countries. This is due in part to storage-induced textural (hard-to-cook) defects and the presence of certain antinutritional factors and nondigestible components. High protein (18−35%) and carbohydrate (50−65%) contents, together with an amino acid pattern complementary to that of cereal grains, however, make cowpeas a potentially important nutritional component in the human diet (Prinyawiwatkul et al., 1996d). Because of the potential of cowpeas as an inexpensive source of significant amounts of protein, calories, and B vitamins such as folacin, niacin, and riboflavin, they should be considered as a valuable food ingredient.

Research has emphasized expanding the utilization of cowpeas in the form of meal and flour (McWatters, 1990) for use as functional ingredients in food products. Increased utility will depend upon development of appropriate technologies to produce meal or flour with acceptable functional properties and enhanced nutritional quality (Prinyawiwatkul et al., 1996d). Dry milling technology that yields a functional, nutritious cowpea flour has been developed (McWatters et al., 1988; Phillips et al., 1988).

Attempts to further enhance the quality of cowpea flour have employed a wide range of technologies, e.g., germination, fermentation, γ-irradiation, and α-galactosidase treatment (Prinyawiwatkul et al., 1996a). *Rhizopus microsporus* subsp. *oligosporus*, the tempeh mold, has been successfully used to ferment partially defatted peanuts which were subsequently milled into flour (Prinyawiwatkul et al., 1993). A simplified solid-substrate fermentation and milling process for preparing flour from nondecorticated cowpeas (cv. White Acre) has been developed (Prinyawiwatkul et al., 1996b). Enhancement of nutritional quality of cowpea flour, including the absence of raffinose and stachyose, increased B-vitamin content, and decreased trypsin inhibitor activity using solid-substrate fermentation with *R. microsporus* has been demonstrated (Prinyawiwatkul et al., 1996a,c). Scale-up production of this flour would stimulate prospects for its utilization.

While nutritional quality is ultimately important in considering cowpea flour as a food ingredient, its successful performance depends largely on functional characteristics imparted to the final products. The versatility of cowpea flour as a base for many food products (McWatters et al., 1995) emphasizes the need for a better understanding of its functional characteristics. Soaking and boiling of cowpeas to be used as a substrate in solid-substrate fermentation are necessary if an acceptable fermented cowpea flour is to be produced; however, these treatments may have a great impact on flour functionality.

The objective of this study was to determine changes in functional properties of cowpea flour as affected by soaking, soaking/boiling (S/B), and solid-substrate fermentation of seeds with *R. microsporus* subsp. *oligosporus* before milling.

MATERIALS AND METHODS

Preparation of Flour from Fermented Cowpeas. Mature dry cowpea seeds (cv. White Acre, 1993 crop) were obtained from Southern Frozen Foods, Montezuma, GA. Upon receipt, cowpeas were visually inspected and defective seeds were discarded. Cowpeas were stored at 7 °C and 60% relative humidity until used.

Cowpeas (1.75 kg) were soaked in tap water (cowpeas:water, 1:6, w/w) at room temperature (ca. 25 °C) for 24 h. Seeds were then boiled in the same soak water for 45 min, drained, cooled to 25−30 °C, and uniformly inoculated with a commercial dried powder *R. microsporus* subsp. *oligosporus* starter culture (Tempeh Lab, Inc., Summertown, TN) at a ratio of 1:200 (starter:cooked cowpeas, w/w). The inoculated seeds (1-kg batch) were placed in perforated plastic Zip-loc vegetable bags (gallon size, 26.8 cm × 27.9 cm, DowBrands L.P., Indianapolis, IN). Bags were placed on a wire mesh screen and incubated at 30 °C for 0 (inoculated but dried immediately), 15, 18, 21,

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and 24 h. Fermented cowpeas were then oven-dried at 60 °C for 13 h and finely ground through a 1-mm screen in a Thomas-Wiley Laboratory Mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA). Cowpea flours were placed in Zip-loc freezer bags, sealed, and stored at −18 °C until used. Two batches of fermented cowpeas were prepared, and flour from both batches was thoroughly mixed before being subjected to analyses.

Nitrogen Solubility Profile. Cowpea proteins were extracted from an 8% (dry w/v) flour suspension in deionized water at room temperature (ca. 25 °C). Slurries were thoroughly mixed for 5 min and adjusted to pH 2.0, 4.0, 6.0, 8.0, and 10.0 by adding appropriate amounts of 1−6N HCl or 1−6N NaOH to avoid substantial increases or differences in final volume. After 1 h of constant mixing at ca. 25 °C using a magnetic stirring bar, the slurry was centrifuged (Model J2-21M, Spinco Division of Beckman Instruments, Inc., Palo Alto, CA) at 15300*g* for 15 min at 4 °C. Supernatant liquid was then filtered through Whatman No. 1 filter paper to obtain a clear extract. Triplicate, 25-mL extracts of flour prepared using each treatment were analyzed for nitrogen content using the Kjeldahl method (AOAC, 1984). Nitrogen solubility (%) was calculated as [(g of N in sample extract × 100)/(g of N in flour sample)]. Flour suspensions with unadjusted (natural) pH values were also subjected to nitrogen solubility determination. The unadjusted pH values of flours made from control, soaked, S/B, and 0-, 15-, 18-, 21-, and 24-h fermented seeds were, respectively, 6.42, 6.41, 6.63, 6.38, 6.42, 6.82, 6.87, and 6.97.

Emulsion Capacity. Oil-in-water emulsion capacity of 8% flour suspensions was determined in triplicate at various pH values using a procedure described by Prinyawiwatkul et al. (1993). Flour slurries were prepared as described for determining the nitrogen solubility profile. Soybean oil (Kroger Co., Cincinnati, OH) supplemented with 0.03% Oil-Red-O biological stain (Aldrich Chemical Co., Inc., Milwaukee, WI) was dispensed from a 100-mL buret through a 2-cm-diameter hole at the bottom of an inverted blender jar. Oil was added dropwise at a rate of ca. 0.4 mL/s with an interruption after 5-mL addition to minimize increases in temperature while the mixture was blended at low speed in an Osterizer blender (Model 869-18R, Oster Division of Sunbeam Corp., Milwaukee, WI). The breakpoint at which phase inversion (coalescence) occurred was considered as the emulsion capacity of flour suspensions. Emulsion capacity was expressed as milliliters of oil emulsified per milligram of soluble protein and as milliliters of oil emulsified per milligram of total protein in 25 mL of flour suspension.

Emulsion Viscosity. Emulsions containing 80% of the amount of oil needed to reach the breakpoint were used for emulsion viscosity measurements (McWatters and Cherry, 1977). Viscosity (cP) was determined at 25 °C using a Brookfield Synchrolectric viscometer (Model RVT, Brookfield Engineering Laboratories, Stoughton, MA) and a Helipath Stand (Model C) equipped with a T-B spindle operated at 2.5 rpm. Five readings were recorded on triplicate samples of emulsions prepared from flour obtained from each treatment. Various emulsion consistencies were described using arbitrary classifications reported by McWatters and Cherry (1977).

Water Adsorption Isotherm. Equilibrium moisture contents (EMC) of cowpea flours at various equilibrium relative humidities (ERH) were determined at 25 °C. Saturated salt solutions were placed in glass desiccators and allowed to equilibrate at 25 °C for 3 days. ERH values were estimated from those reported by Rockland (1960): LiCl (11%), $MgCl_2$ (33%), $Mg(NO_3)_2$ (52%), NaCl (75%), and K_2SO_4 (97%). Triplicate, ca. 3-g samples of oven-dried flours were weighed into tared aluminum pans and placed above saturated salt solutions in closed desiccators. After 15 days, samples were weighed and the EMC of flours was calculated.

Water and Oil Retention. Cowpea flours (5.0 g) were thoroughly mixed, without pH adjustment, with 25 mL of deionized water or oil in 50-mL centrifuge tubes. Suspensions were stirred intermittently over a 30-min period at 25 °C and then centrifuged at 12000*g* for 30 min at 25 °C. The volume of decanted supernate was measured, and the water and oil retention capacities were calculated. Triplicate samples were analyzed for each flour.

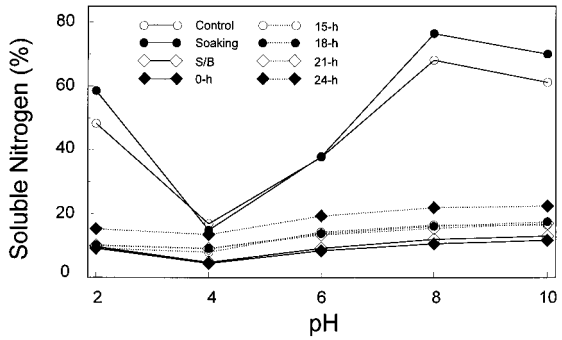

Figure 1. Nitrogen solubility profiles of cowpea flours as affected by soaking, soaking/boiling (S/B), and fermentation for up to 24 h.

Least Gelation Capacity. Triplicate suspensions of 1−20% cowpea flour (dry w/v, at 1% increments) were prepared in 10 mL of deionized water and mixed thoroughly without pH adjustment. The slurries were heated in 125 × 20 mm screw-capped test tubes in a water bath at 95 ± 5 °C with intermittent stirring. After 1 h of heating, tubes were immediately cooled in tap water for 30 s and then in ice water for 5 min to accelerate gel formation. All tubes were then held at 4 °C for 3 h. Least gelation capacity (percent) was determined as the concentration above which the sample remained in the bottom of the inverted tube.

Statistical Analysis. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's studentized range test for post-hoc multiple comparisons (SAS, 1988).

RESULTS AND DISCUSSION

Nitrogen Solubility Profile. Among the functional properties of proteins, solubility is probably the most critical because it affects other properties such as emulsification, foaming, and gelation (Kinsella, 1976). Nitrogen solubility profiles of flours prepared from control and soaked White Acre seeds (Figure 1) are typical of those reported for other cowpea cultivars (Okaka and Potter, 1979; Schaffner and Beuchat, 1986; Sosulski et al., 1987; Abbey and Ibeh, 1988; Giami, 1993). Solubility of unheated protein in flours prepared from control and soaked seeds as affected by pH was somewhat higher than that reported for California black-eyed 5 cowpeas (Schaffner and Beuchat, 1986) but somewhat lower than for Texas Cream 40 cowpeas (Sosulski et al., 1987). The variations exist due mainly to cultivar differences and processing and sample preparation.

Solubility of cowpea proteins is greater at alkaline than at acidic pH. In general, increasing pH from 4.0 to 8.0 considerably increased protein solubility of flours made from control and soaked seeds (Figure 1). An increase in pH from 8.0 to 10.0, however, did not increase solubility. As in most legumes, minimum solubility occurs near the isoelectric point (p*I* ≈ 4.0) of proteins. At pH 4.0, about 15−17% of cowpea proteins were soluble and thus extractable (Figure 1). A solubility range of 17−40% of unheated cowpea proteins at their p*I* has been reported (Okaka and Potter, 1979; Sefa-Dedeh and Stanley, 1979a; Padmashree et al., 1987; Sosulski et al., 1987). Unlike cowpeas, most legumes have a protein solubility of about 10% or less at their p*I* (Sefa-Dedeh and Stanley, 1979a). The higher extraction of proteins at pH near their p*I* may be due to their composition. Aggregation of cowpea proteins is not necessarily accompanied by complete insolubili-

zation, as some albumins and globulins may not precipitate at their p*I*. For example, the 7−8-S fraction (vicilin) of cowpea proteins has been shown to be soluble, even at the p*I* value (Sefa-Dedeh and Stanley, 1979b).

Solubility of most proteins is markedly and irreversibly reduced when severe heat treatment is applied. Boiling cowpeas for 45 min before flour preparation caused protein denaturation and thus a drastic decrease in solubility (Figure 1). This was due to the exposure of hydrophobic groups and to the aggregation of the unfolded protein molecules. Reduction in protein solubility of unheated flour prepared from control or soaked seeds (50.6−54.2%) compared to that (8.8%) of flour made from S/B seeds was as high as 83.8% at unadjusted pH values. The negative effect of heat treatment on solubility of cowpea flour proteins has been reported by other researchers (Enwere and Ngoddy, 1986; Padmashree et al., 1987; Abbey and Ibeh, 1988; Phillips et al., 1988; Giami, 1993).

Nitrogen solubility profiles of flours prepared from fermented seeds (Figure 1) differ from those reported for other types of fermented plant proteins (Quinn and Beuchat, 1975; Shieh et al., 1982; Canella et al., 1984). Schaffner and Beuchat (1986) reported a reduction in solubility of cowpea protein over a wide pH range as a result of fermentation. The flat solubility curve for cowpea protein at pH 2−10 caused by heat treatment was observed in the present study. Because the process of preparing fermented cowpea powder (Schaffner and Beuchat, 1986) involved heat treatment (121 °C, 10 min), it is more likely that reduced solubility was due to protein denaturation by heat than by fermentation.

Solubility of denatured proteins can be increased by bacterial and fungal protease activity (Bernardi Don et al., 1991). Denaturation is known to increase susceptibility of proteins to degradation by protease due to the unmasking of peptide bonds (Cheftel et al., 1985). Fungal fermentation (15−24 h) slightly increased solubility of heat-denatured proteins at all pH values investigated (Figure 1). For instance, at pH 4.0, the solubility of flour increased (205%) from 4.4% at 0 h of fermentation to 13.4% after 24 h of fermentation. An increase in solubility (128%) from 8.2% for 0-h-fermented flour (pH 6.63) to 18.7% for 24-h-fermented flour (pH 6.97) occurred at unadjusted pH values. Reduction in molecular size and exposure of hydrophilic sites due to fungal protease cleavage may have been responsible for the higher solubility.

Although loss of solubility caused by heating can be recovered by fungal fermentation, the recovery did not reach the original solubility level (Figure 1), under the conditions used in this study. Reduced solubility caused by heat treatment was evident even after 24 h of fermentation. Reports on increased nitrogen solubility of legume and oilseed flours caused by natural, lactic acid bacterial or fungal fermentation indicate a lack of general agreement (Canella et al., 1984; Schaffner and Beuchat, 1986; Padmashree et al., 1987; Giami, 1993; Prinyawiwatkul et al., 1993). One explanation for these conflicting observations is that solubility is generally improved if severe heat treatment is not involved in the fermentation process.

Emulsion Capacity (EC) and Viscosity (EV). Emulsion characteristics of proteins contribute much to the functionality of foods. The emulsifying properties of cowpea flour proteins are pH (solubility) dependent (Figure 2). Soluble proteins are inherently surface active due to their amphiphilic nature and tendency to adsorb at oil−water interfaces. The EC profiles of cowpea flours expressed as a function of pH and soluble protein (Figure 2a) and total protein (Figure 2b) are different. On the basis of total protein content, EC values at pH 2.0−10.0 were relatively constant (part b vs part a of Figure 2). It has been hypothesized that emulsifying properties are influenced more by quality than quantity of soluble proteins (Prinyawiwatkul et al., 1993). This is substantiated by observations in the present study. For example, at pH 4.0, there was no emulsion formed for 24-h-fermented flour (71.9 mg of soluble protein/25 mL of flour suspension), but there was an emulsion formed for flour made from soaked seeds (72.5 mg of soluble protein). Further evidence is seen at pH 10.0, at which emulsion formation was observed for heated, unfermented (0 h) flour (60.6 mg of soluble protein), but no emulsion was formed from fermented (24-h) flour, which contained almost twice as much soluble protein as 0-h-fermented flour.

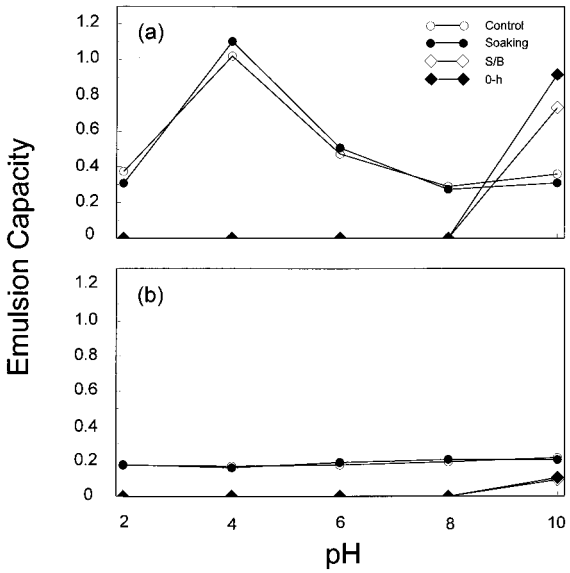

Figure 2. Emulsion capacity of cowpea flours as affected by pH: (a) milliliters of oil emulsified per milligram of soluble protein; (b) milliliters of oil emulsified per milligram of total protein in 25 mL of flour suspension. See Figure 1 for symbols.

Because the EC values are expressed on a per milligram (soluble or total) of protein basis, they decrease with increased protein concentration, as clearly demonstrated in Figure 2a vs Figure 1. Consequently, the assumption that proteins must have high initial solubility as a prerequisite for emulsifying properties does not always hold. Indeed, a positive correlation between solubility and EC of flours made from unheated control and soaked seeds does not exist. High solubility at pH 2.0, 8.0, and 10.0 was not proportionately correlated with increased EC values (Figure 2a vs Figure 1). On the basis of soluble protein content, the highest EC value (82 mL of oil/80.7 mg of protein) of control flour was observed at pH 4.0, although the highest amount of oil emulsified (105 mL of oil/292.8 mg of protein) was at pH 10.0 (Figure 2a). Experimental data are conflicting in that some proteins have optimal emulsifying properties at their p*I* and others perform better at pH values away from the p*I* (Cheftel et al., 1985). Emulsion formation of flours from unheated control and soaked seeds at the p*I* observed in this study (Figure 2a) can be rationalized. First, a monolayer film at the oil−water interface can occur at very low protein concentration (Narsimhan, 1992). This results in fairly high resistance to coalescence of emulsion droplets

under normal conditions (Halling, 1981). An aforementioned observation was that about 15−17% of unheated cowpea proteins were soluble at their p*I*. Perhaps this amount of soluble protein was sufficient for emulsion formation. Second, proteins exhibit compact structures with high viscoelasticity at their p*I*; this may either prevent unfolding and adsorption at the oil−water interface or stabilize an adsorbed protein film against surface deformation (Cheftel et al., 1985). It is more likely that cohesiveness and rigidity of cowpea proteins at their p*I* further stabilized the existing protein film against droplet coalescence.

No emulsion was observed (except at pH 10.0) for flours prepared from S/B and unfermented (0 h) seeds (Figure 2). Heat treatment (boiling for 45 min) of seeds sharply reduced solubility and impaired emulsifying properties of flours. The solubility of flours from S/B and 0-h-fermented seeds was 11.6−12.9% at pH 10.0. The lower solubility at pH 2.0−8.0 may have been responsible for the inability of heated, nonfermented flours to form emulsions. Heating causes protein denaturation but is not always accompanied by loss of emulsifying properties of cowpea proteins (Aluko and Yada, 1993). This is because the formation of an emulsion involves various degrees of protein unfolding, aggregation, and insolubilization (Cheftel et al., 1985). Mild heat treatment (100 °C, 3 min) has been reported to increase emulsifying properties of peanut flour (Prinyawiwatkul et al., 1993). Improvement was probably due to dissociation and partial unfolding of globular proteins to expose the hydrophobic sites of amino acids, which consequently increase surface activity and adsorption at the oil−water interface (Voutsinas et al., 1983; Nir et al., 1994). Severe heat treatment yields undissolved, denatured proteins that are unable to orient and migrate to form an interfacial film at the oil−water interface, thus preventing emulsion formation, as is the case in this study.

Regardless of pH, emulsions were not formed from flours made from heated fermented cowpeas. This was undoubtedly due to heat denaturation of proteins. Increased solubility as a result of *R. microsporus* protease activity after 24 h of fermentation did not restore the emulsifying properties impaired by heat treatment. The effects of nonprotein constituents in flour made from whole seeds are not known. Cowpeas contain 50−60% dietary carbohydrate, of which starch contributes about 60−77% (Longe, 1980). Starch is not a good emulsifying agent, primarily because it lacks polyelectrolyte character necessary for emulsion stability (Pomeranz, 1991). The presence of carbohydrate and fiber in vegetable protein flour was reported to adversely affect EC (Ramanatham et al., 1978).

The EV profiles of flours from control and soaked seeds (Figure 3) were more similar to nitrogen solubility profiles (Figure 1) than EC profiles (Figure 2). Data (Figures 1, 2a, and 3) indicate that high EC is not necessarily associated with high EV or nitrogen solubility. Flours from control and soaked seeds exhibited low EV at pH 4.0−6.0 but high EV at pH 2.0, 8.0, and 10.0. In flour with high EV, inversion may not lead immediately to a continuous oil phase but rather to an oil−water−oil double emulsion. Similar to changes in EC, heat treatment resulted in no measurable EV of flours prepared from S/B and 0-h-fermented seeds.

Classification of food emulsions is often based on the dispersion phase volume ($\varnothing = V_i/[V_i + V_e]$, where V_i and V_e are, respectively, internal and external phase volume). Emulsions formed by flours made from control and soaked seeds had calculated $\varnothing$ values ranging from 0.76 to 0.81 at pH 2.0−10.0. Lynch and Griffin (1974) noted that emulsions having $\varnothing > 0.7$ are called high internal phase ratio emulsions, and foods such as mayonnaise and salad dressings fall into this category. On the basis of arbitrary viscosity values reported by McWatters and Cherry (1977), a similar classification could be obtained for emulsions evaluated in our study. Semithick mayonnaise-like emulsions were produced at pH 2.0 and 10.0, and very thick salad-dressing-like emulsions were produced at pH 8.0. Results indicate that the capacity of unheated cowpea flour proteins to contribute to the formation of emulsions may be desirable for many applications such as comminuted meats, cake batters, mayonnaise, and salad dressings.

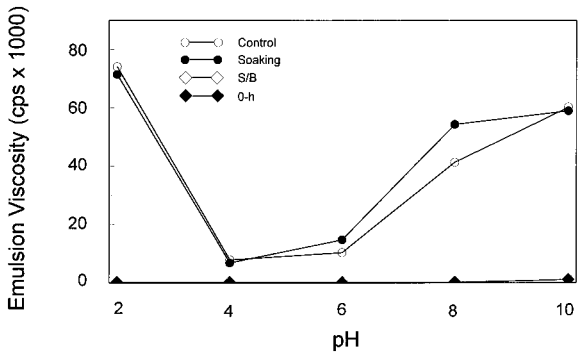

Figure 3. Emulsion viscosity of cowpea flours as affected by pH. See Figure 1 for symbols.

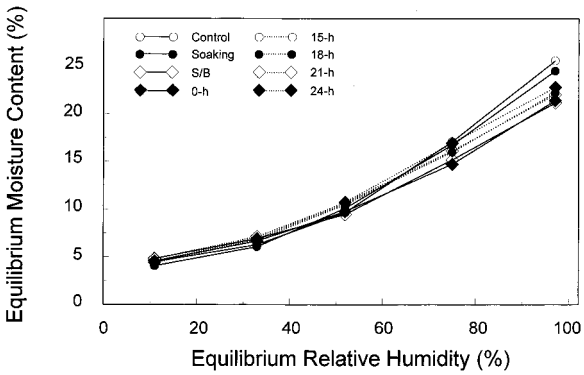

Figure 4. Equilibrium moisture content of cowpea flours as affected by relative humidity at 25 °C. See Figure 1 for symbols.

Water Adsorption Isotherm. Water adsorption isotherms of nonfermented and fermented cowpea flours (Figure 4) are similar to those of dry- and wet-dehulled cowpea flours reported by Chhinnan and Beuchat (1985). The combined effects of heat treatment and fungal fermentation on the EMC were minor at ERH but were more obvious at higher ERH. The EMC of flours stored at 11% ERH ($a_w = 0.11$) ranged from 4.05 to 4.81%. The capacity of fermented cowpea flours to bind a greater amount of water without substantial change in a_w in the 33−52% ERH range has practical implications when one is formulating intermediate moisture foods, especially at $a_w = 0.60$, a level regarded as minimum for fungal growth in foods (Beuchat and Hocking, 1990). The EMC of cowpea flours that would result in a_w values of 0.52 and 0.75 are, respectively, ca. 10−11% and 17%. This EMC range could be used as an indicator of the likelihood that cowpea flours will undergo fungal deterioration during storage (Chhinnan and Beuchat, 1985).

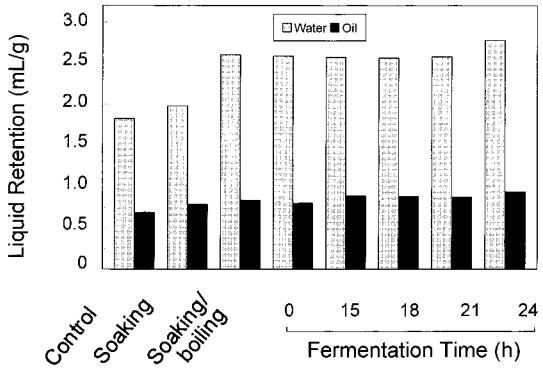

Figure 5. Water and oil retention of cowpea flours.

Water and Oil Retention. Water retention capacity of cream-type cowpea flours was 1.71 g/g for Texas Cream 12 (Prinyawiwatkul et al., 1994) and 1.82 g/g for White Acre (Figure 5). The slightly higher water retention of flour from soaked seeds (1.97 g/g) compared to that of control seeds (1.82 g/g) was likely due to greater starch swelling (Okaka and Potter, 1979; Prinyawiwatkul et al., 1996e). Heat-processed (S/B) flour had greater water retention than did flours from control and soaked seeds. The increase may have been due to changes in concentration and structural conformation of proteins.

There was no correlation between solubility at unadjusted pH values (Figure 1) and water retention (Figure 5) of cowpea flours; that is, increased protein solubility did not necessarily yield increased water retention capacity. Hermansson (1979) noted that solubility values give no information concerning the extent to which proteins can bind water. Total protein content, however, has been reported to be primarily responsible for increased water retention (Hutton and Campbell, 1981). Because the protein contents of flours made from control (26.2%), soaked (26.7%), S/B (27.1%), and 24-h-fermented (28%) seeds were not substantially different (Prinyawiwatkul et al., 1996b), the increased water retention observed in this study may not be due to the total protein content.

Cheftel et al. (1985) noted that water absorption of proteins can be improved by partial denaturation, dissociation, unfolding, and insolubilization. Improvement of water retention of cowpea flour by heat treatment has been reported (Padmashree et al., 1987; Abbey and Ibeh, 1988; Phillips et al., 1988; Giami, 1993). Insolubilization of heat-denatured protein increased water retention of cowpea flour; however, protein solubility changes did not correspond to water retention capacity (Phillips et al., 1988). Boiling cowpeas for 45 min during flour preparation may have caused conformational changes of proteins from globular to random coil, exposing buried amino acid side chains, thereby making them available to interact with water (Hutton and Campbell, 1981). Fermentation for up to 24 h did not greatly affect water retention capacity of flour prepared from boiled, nonfermented (0 h) cowpeas (Figure 5), although an increase in polar groups ($-NH_4^+$ and $-CO_2^-$) as a result of *R. microsporus* proteolytic activity is known to increase the hydrophilicity of proteins (Prinyawiwatkul et al., 1993).

Protein−water interactions occur at polar amino acid sites on the protein molecules. Most proteins contain numerous polar side chains along their peptide backbone, making them hydrophilic. Regardless of soaking treatment (Okaka and Potter, 1979), mode of heat treatment (Padmashree et al., 1987), or flour particle size (Ward, 1995), cowpea flour is more hydrophilic than lipophilic. Results from our study (Figure 5) also indicated that cowpea flours were consistently more hydrophilic than lipophilic, regardless of processing treatment. The oil retention capacity of cowpea flours ranged from 0.69 to 0.93 g/g. Slight increases in oil retention were observed as a result of boiling and fungal fermentation. The effects of heat treatment on oil retention of flours were less compared to water retention. The majority of reports on water and oil retention of plant protein additives in food systems have involved their incorporation into comminuted meat systems (Hutton and Campbell, 1981). The ability of cowpea flours to absorb and retain water and oil may help improve binding of the structure, enhance flavor retention, improve mouthfeel, and reduce moisture and fat losses of extended meat products (McWatters, 1977; McWatters and Heaton, 1979).

Least Gelation Capacity. The critical factor influencing gel formation is the protein concentration. Below a minimum concentration, aggregation and increased viscosity of cowpea flour suspensions were observed, but gelation did not occur. At least 10% (dry w/v) control flour (ca. 2.6% protein) was required for gel formation. A least gelation capacity range of 5.5−16% for raw cowpea flour has been reported (Enwere and Ngoddy, 1986; Abbey and Ibeh, 1988; Olaofe et al., 1993). Gelation takes place more readily at higher protein concentration because of the greater intermolecular contacts during heating. High protein solubility is not always necessary for gelation, as observed in this study.

Heat treatment increased the least gelation capacity of cowpea flours. At least 15% (dry w/v) heated (S/B) flour was required compared to 10% control flour for gel formation. Because cowpeas contain high protein and starch content, the least gelation capacity of flours is influenced by a physical competition for water between protein gelation and starch gelatinization. The major difference between flours made from control and soaked seeds and flours made from S/B seeds is the functional qualities of protein and starch. After cowpeas were boiled for 45 min, proteins were undoubtedly denatured, unfolded, and aggregated. Prinyawiwatkul et al. (1996e) reported that almost complete gelatinization of starch occurred during boiling of cowpeas (45 min), as evidenced by a loss of starch birefringence characteristics. Furthermore, on the basis of viscoamylogram data, the ability of preheated flours to form a viscous paste and to form a gel upon cooling was due to protein gelation rather than starch gelatinization (Prinyawiwatkul et al., 1996e). This suggests that flour with high protein (which has been denatured) and starch (which has been pregelatinized) contents would require greater amounts of flour before thermal gel formation could occur. The lower amounts of flours from control and soaked seeds required for gel formation are therefore due to synergistic effects of protein and starch. Fermented (15−24 h) flours had slightly lower least gelation capacity (14%) than that (15%) of heated, nonfermented (0 h) flours; this was likely due to their higher water retention capacity (Figure 5). The ability of cowpea flours to absorb/retain water and oil and to form a gel is desirable for the preparation of various comminuted meat or fish products.

In summary, this study has shown that functional properties of cowpea flours are greatly affected by various processing treatments administered during flour

preparation. Soaking and fungal fermentation had less impact than boiling on flour functionality. An understanding of changes in functional properties as influenced by these treatments will be of value in determining specific end uses of cowpea flour. The ability of unheated cowpea flour to enhance the formation of emulsions would be desirable for applications such as sausages, mayonnaise, and salad dressings. Heat treatment impaired emulsifying properties of flours. However, the ability of flours made from heated seeds to absorb and retain water and oil and to form a gel-like structure would be desirable in the preparation of extended meat products in which quality is not dependent on emulsifying properties. Potential applications of cowpea flours in other products such as bakery and extruded snack foods are also numerous and need further investigation.

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